Slide 1

Interferon Gamma Release Assays and the Diagnosis of Latent Tuberculosis

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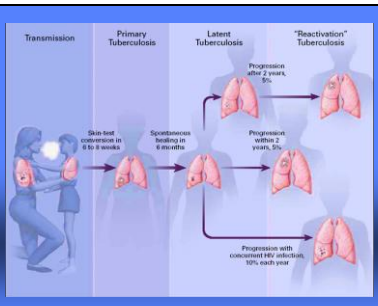
Mississippi State Department of Health

Slide 2



Slide 3

Transmission

- Small droplets (<100µm) coughed up by pulmonary TB patients aerosolize and float through the air
- These droplets fall to the ground faster in humid conditions
- Small droplets containing TB bacilli settle in lung alveoli
- Bacilli are ingested by alveolar macrophages

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Survival and Proliferation

- Bacilli survive and proliferate within macrophages
- Bacilli kill macrophages, are ingested by new macrophages and continue to proliferate
- Bacilli spread to lymph nodes and spread systemically

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Host Immune Response

- Cell mediated immunity (T-Cell directed) coordinates immune response
- Immune system contains/limits bacilli growth

OR

- There is an ineffective immune response and the patient progresses to primary disease

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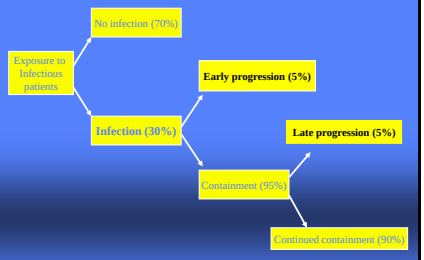

Natural History of TB Infection

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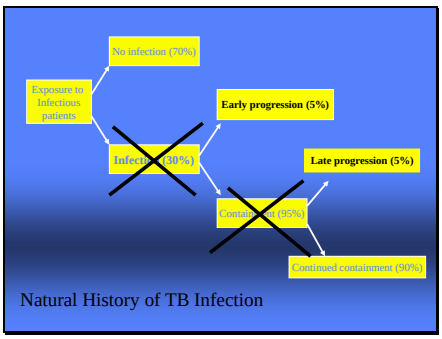

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Diagnosis of TB: Purified Protein Derivative

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Brief History of PPD

- Tuberculin – developed by Robert Koch 1890's as "therapeutic agent"
- Heat sterilized extract of TB proteins
- Ineffective as a treatment but diagnostic value appreciated (Old Tuberculin)

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PPD

- Purified Protein Derivative – developed in 1939 by Florence Siebert in Philadelphia
- Precipitate prepared by filtration of Old Tuberculin
- Mixture of ~170 different proteins
- Intradermal injection leads to delayed type hypersensitivity for those with prior exposure

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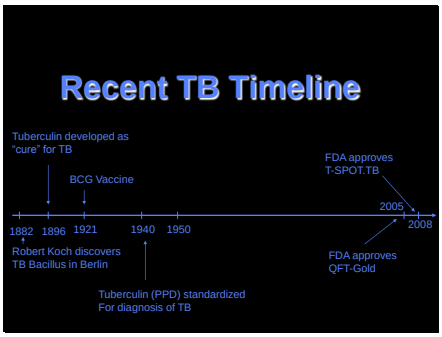

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Diagnosing Latent TB

- Tuberculin Skin Testing
- IGRA's (Quantiferon and T-spot)
- Do not differentiate between Latent and Active Disease!!!

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Testing for *M. tuberculosis* Infection

- **Mantoux tuberculin skin test (TST)**
  - Skin test that produces delayed-type hypersensitivity reaction in persons with *M. tuberculosis* infection
- **Interferon-gamma Release Assays**
  - Blood tests that measures and compare amount of interferon-gamma (IFN-γ) released by blood cells in response to antigens
    - QuantiFERON® TB tests
      - QuantiFeron Gold
      - Quantiferon Gold-IT
    - T-SPOT.TB

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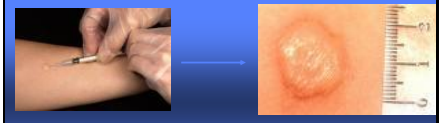
Tuberculin Skin Test



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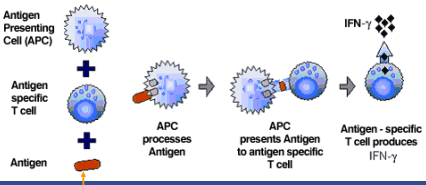
IGRA's



ESAT 6, CFP 10, TB7.7 - antigens quite specific to TB (not on M avium or BCG)

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Purpose of Targeted Testing

- Find persons with LTBI who would benefit from treatment to prevent disease
- Find persons with TB disease so that treatment can be started

Groups that are not at high risk for TB should not be tested routinely

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IGRA

- **Quantiferon**
- **T-spot**

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Brief History of IGRA's

- **Quantiferon – initially developed as a test for Bovine TB in Cattle**
- **Whole blood incubated with PPD for 16-24 hours**

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Evolution of Quantiferon Assay

- QFT - Whole blood incubated with PPD
- QFT Gold – Whole blood incubated with TB antigens ESAT-6 and CFP-10
- QFT Gold In Tube - Whole blood incubated with TB antigens ESAT-6, CFP-10 and TB 7.7

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How do IGRA's Differ from TST

- TST – nonspecific extract of attenuated MTB strain
- QFT Gold IT – ESAT-6, CFP-10, TB 7.7
- T-Spot – ESAT-6, CFP-10

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Problems with TST

- Non-specific for MTB (other NTM's and BCG)
- Subjectivity of Reading
- Second visit required

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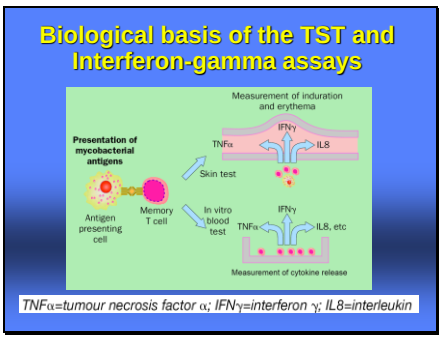

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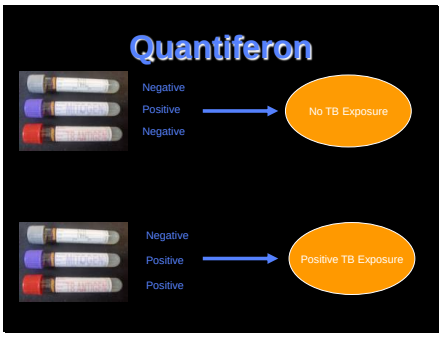

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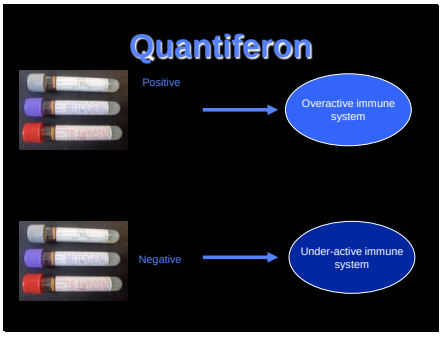

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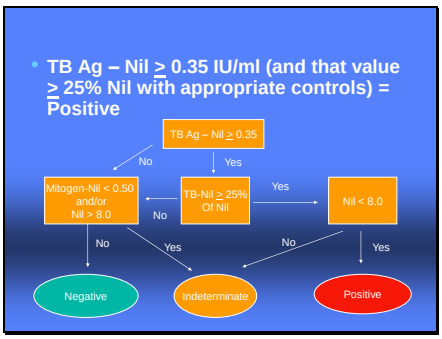

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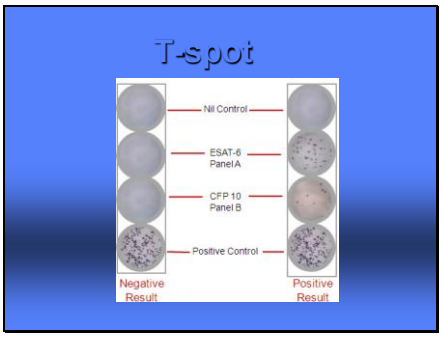

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Methodology of T-Spot

- 250,000 Peripheral Blood Mononuclear Cells are collected from whole blood and placed in well
- Cells producing Gamma-interferon due to TB antigen exposure counted and compared to controls
- Benefit of standardization of cell numbers

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Sensitivity of IGRA

- Meta-analysis*:
  - Elispot: 88%
  - QFT: 76%
  - TST: 70%
- IGRA's possibly more sensitive in immunocompromised

* Menzies, et al. Ann Intern Med 2007; 146:340.

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Enhanced Specificty vs. TST

- NTM (MAC) –

(with exception of *M kansasii, M szulgai, M. marinum*)

- BCG

Slide 30

Int J Tuberc Lung Dis. 2001 Dec;5(12):1122-8.

Skin test reactions to Mycobacterium tuberculosis purified protein derivative and Mycobacterium avium sensitin among health care workers and medical students in the United States.

van Reyn CF, Horsburgh CR, Olivier KN, Barnes PF, Waddell R, Warren C, Tvaroha S, Haegni AS, Lein AD, Alexander TN, Weiner DJ, Tosteson AN.

- Dual skin testing was performed with PPD and Mycobacterium avium sensitin on 784 health care workers and medical students in the northern and southern US.
- CONCLUSIONS: Infections with NTM are responsible for the majority of 5-14 mm PPD reactions among US-born health care workers and medical students subject to annual tuberculin testing.

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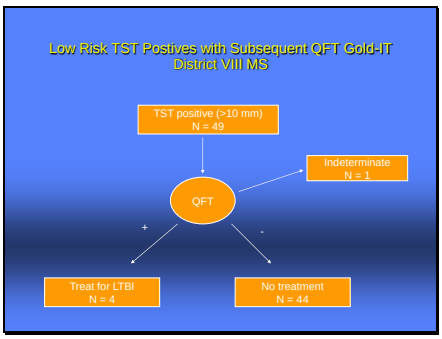

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IGRA's

- The problems related to IGRA's include:
  - Cost of the test kits
  - Equipment
  - Personnel
  - Need for blood drawing
  - Time barrier for specimen processing and analysis
- Benefits
  - No need for return to clinic
    - Shelters, prisons & jails
  - No false positive from prior BCG vaccination or most NTM's
  - Non-subjective Interpretation (inter-reader variability)

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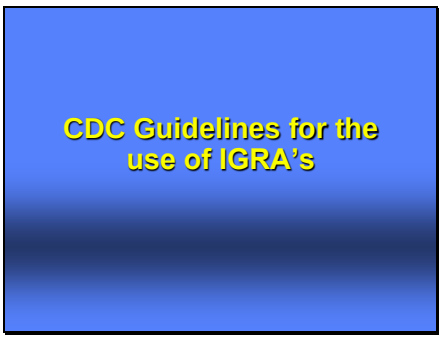
Time Barriers for IGRA's

- Quantiferon –
  - Must incubate within 16 hours of collection
  - Incubation 37C 16-24hrs
  - After incubation, may store up to 72 hours (2C-27C)
- T-Spot – Must Process within 8 hours of collection (32 hours if treated with T-cell Extend)

Slide 34



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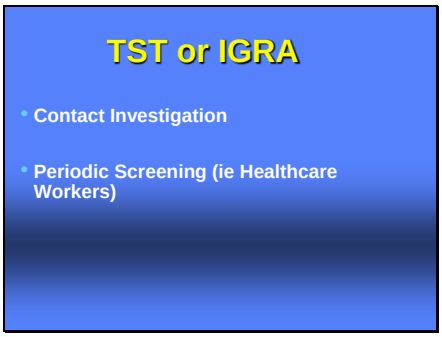

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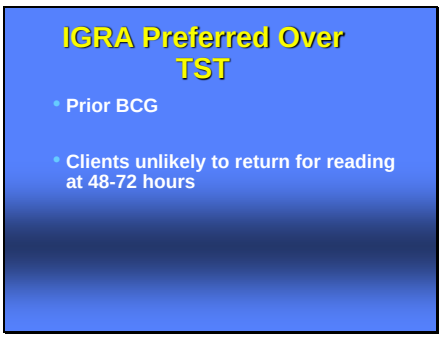

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TST Preferred Over IGRA

- Children < 5 years old

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IGRA and TST May Be Considered

- Improve Sensitivity
  - High risk individuals (contacts <5yo)
  - TB Suspects
- Improve Specificity
  - Low risk TST positive
- Improve Accuracy
  - TST when IGRA result borderline/high nil (or repeat IGRA)
- Improve Acceptance/Compliance
  - Foreign Born with prior BCG

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Canadian Guidelines for Use of IGRA's

2008

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- Similar to CDC Guidelines
- Do not endorse IGRA's for Serial testing or Children < 18 (2008 Recs though)
- *Suggest IGRA for confirmation of positive TST in low risk individuals including low risk contacts*

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Use of IGRA's in Immunocompromised

- HIV – Correlates better with Risk Factors for LTBI than
  - Higher rate of "Indeterminate" results when CD4 < 100
- Immunosupressive Rx –
  - TST-/IGRA+ discordance with steroids
  - IGRA better assoc with TB Risks

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Use of IGRA's in Children

- Little performance data for children < 5
- Higher proportion of indeterminate results in those < 5 (usually low mitogen response)
- TST recommeded for children < 5

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Risk of TB with Negative IGRA

- Good negative predictive value – (100% TB contacts with TST positive and negative QFT) (1,2)
- No difference in predictive value in other studies (3)

1. Diel R, et al *Am J Respir Crit Care Med 2008; 177:1164–70.*
2. Higuchi K, et al *Respirology 2007; 12:88–92.*
3. Kik SV, et al. *Eur Respir J 2009.*

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Use of IGRA's in Setting of Repeat Annual Testing

- IGRA better correlated to risk
- Lower number of IGRA+ than TST (except in high incidence settings)
- IGRA known to have slight variation on sequential testing with "reversions" to normal

1. Zwerling A, et al. Thorax. 2011(Epub)
2. Pai M, etal. Am J Respir Crit Care Med 2006;174:349–55.

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Case Example

- 62 yo hospital transcriptionist
- TST neg since 2002
- No known TB exposure
- 2008 – QFT - *Positive*

0.16 IU/ml

0.67 IU/ml

8.71 IU/ml

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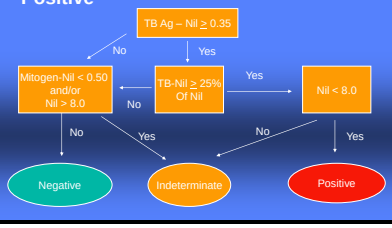
- TB Ag – Nil ≥ 0.35 IU/ml (and that value ≥ 25% Nil with appropriate controls) = Positive



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Case Example

- TST negative
- Repeat QFT – 2 weeks – Negative
- Repeat QFT – 1 year - Negative

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Cost Effective?

- Oxlade O, Schwartzman K, Menzies D. Interferon-gamma release assays and TB screening in high-income countries: a cost-effectiveness analysis. *Int J Tuberc Lung Dis 2007; 11: 16-26.*
- Diel R, Nienhaus A, Lange C, Schaberg T. Cost optimization of screening for latent tuberculosis in close contacts. *Eur Respir J 2006; 28: 35-44.*
- de Perio MA, Tsevat J, Roselle GA, Kralovic SM, Eckman MH. Cost-effectiveness of Interferon Gamma Release Assays vs Tuberculin Skin Tests in Health Care Workers. *Arch Intern Med 2009; 169: 179-18.*
- Mori T, Harada N. [Cost-effectiveness analysis of QuantiFERON-TB 2nd generation used for detection of tuberculosis infection in contact investigations]. *Kekkaku 2005; 80: 675-86.*

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